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THE TRANSPORT OF Zn^{2+}, Co^{2+} AND Ni^{2+} INTO YEAST CELLS

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SUMMARY

Ni^{2+}, Co^{2+}, Zn^{2+} can be taken up into a non-exchangeable pool by yeast cells, by a system that also transports Mg^{2+} and Mn^{2+}. The affinity series is Mg^{2+}, Co^{2+}, $Zn^{2+} > Mn^{2+} > Ni^{2+} > Ca^{2+} > Sr^{2+}$. The uptake is small in starved cells, but is enhanced in the presence of glucose. It is remarkably stimulated (5–20 fold) if cells are pretreated with phosphate and glucose. The uptakes are the same under aerobic or anaerobic conditions suggesting that fermentative reactions can supply the energy for transport. Uptake is reduced at low pH (below 5.0), but a H^+ exchange system is not involved. Instead, 2 K^+ (or 2 Na^+ in Na^+-loaded cells) are secreted for each divalent cation absorbed.

INTRODUCTION

Two distinct interactions of divalent cations with the cell membrane of yeast cells have been characterized. The first is a binding to fixed anionic groups of the cell surface[1]. The binding follows simple derivations of the mass law, with rapid and complete exchangeability, little discrimination between many divalent cations, for example Mg^{2+}, Mn^{2+}, Ca^{2+}, and Sr^{2+}, and little or no influence on the metabolic state of the cell. The second interaction is with a specific transport system which transfers the cations into the cell into a virtually non-exchangeable pool[2]. The system displays a high degree of specificity for Mg^{2+} and Mn^{2+} over Ca^{2+} and Sr^{2+}, is dependent on metabolic factors, and is markedly enhanced in cells that have been allowed to absorb phosphate[3].

In the case of UO_2^{2+}, interactions with yeast cells are restricted almost completely to binding by anionic groups at the outer surface of the cell, with no measurable transport into the cell. Its inhibitory effect on sugar metabolism can therefore be attributed exclusively to the binding reaction at the membrane, leading to the conclusions that the binding groups are directly involved in sugar transport[4]. Other divalent cations such as Ni^{2+} and Co^{2+} also bind to the surface anions of yeast[5] and interesting relationships to sugar metabolism have been reported[6,7] that have important implications concerning the mechanism of sugar transport. The possibility that these cations can be transported into the cell has not, however, been adequately

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explored. In the present paper it is demonstrated that such a transport can occur, and certain properties of the transport system are described. In another study, it was demonstrated that the transport of Ni^{2+} leads to a partial inhibition of sugar fermentation by specific interactions with the alcohol dehydrogenase system[8].

METHODS

Commercial fresh bakers' yeast (Standard Brands) was starved under aeration for 12 h and washed three times with distilled water. The washed yeast cells were stored at 4° and used for experiments for about 6 days. Before each experiment they were washed again three times and the amount of cells was measured by a cytocrit method involving centrifugation for 8 min in a Wintrobe tube. Because yeast cells treated with glucose and phosphate can shrink considerably, volume measurements were made prior to such treatment. Yeast cells pack upon centrifugation, almost like spheres, with a trapped extracellular volume of 22 % (ref. 9).

The cells were incubated for appropriate times with substrates and salts. Because the cations may precipitate in phosphate buffers and are chelated by many organic anion buffers, the pH was kept constant by statting an unbuffered suspension with 1 M NaOH or 1 M HCl using an automatic titrator (Radiometer, Copenhagen). The small amount of sodium added did not interfere with the uptake of the divalent cations in the cells. At the end of the incubation period, the cells were separated from the supernatant on a millipore filter.

Nickel, manganese, cobalt and zinc were measured by isotope techniques using ^{63}Ni, ^{54}Mn, ^{60}Co, and ^{65}Zn. The amount of radioactive cations taken up from the incubation medium was determined either by the disappearance of radioactivity from the cell-free solution or by directly measuring the radioactivity in the cells. The divalent cations bound to the surface of the cells were displaced prior to separation of the cells by adding a surplus of the particular non-radioactive salt to the medium, or by washing the cells on filter with the non-radioactive salt. The procedure removed all of the exchangeable isotope. That remaining represents the non-exchangeable fraction assumed to be cation transported into the cell. Because Zn^{2+} has a tendency to precipitate in the cell wall space, cells exposed to Zn^{2+} were washed with a non-radioactive zinc solution at pH 3, thus dissolving the precipitate. Potassium and sodium were determined by flame photometric technique.

RESULTS

Ni^{2+}, Co^{2+}, and Zn^{2+} can be taken up by yeast into a non-exchangeable form but the amount of uptake and the rate of uptake are conditioned by external factors, such as the pH, the cation concentration, and the temperature, and by internal factors such as the activity of the metabolic system and pretreatment with substrate and phosphate.

In well-starved cells without substrate, small but significant amounts of Co^{2+} and Zn^{2+} were taken up, about 0.4 mmole/kg yeast (wet wt.) mostly within the first 5 min of exposure, but no significant uptake of Ni^{2+} could be measured. The addition of glucose resulted in an increased uptake of cations, particularly Co^{2+} and Ni^{2+}. The rate of uptake was 0.1 to 0.2 mmole/kg per min and it was maintained for longer

periods of time; the total uptake exceeded 1–2 mmoles/kg. The rate of uptake was the same in the presence and absence of O_2. The most dramatic effect, however, was observed in cells pretreated with glucose and phosphate, but washed free of residual phosphate before testing for divalent cation uptake. The rates increased as much as 20 fold. The time course of the effect of pretreatment is demonstrated in Fig. 1 for the uptake of Ni^{2+} in the presence of glucose and of Zn^{2+} in the absence of glucose. In untreated cells the uptakes were 0.1 mmole/kg per min and 0.05 mmole/kg per min, respectively. After a lag period of 10–20 min the pretreated cells reached levels of uptake of 0.5 and 0.9 mmole/kg per min, increases of 5 fold and 18 fold. Furthermore, the uptake was maintained with only gradual falling off for over 20 min making the total uptake greater than 7 mmoles/kg (Fig. 2).

The rate of cation uptake is dependent on extracellular pH. Below pH 5 the rate for Ni^{2+} uptake was reduced considerably, with the midpoint of the effect at about pH 4.3 (Fig. 3). The uptake of the other cations showed a similar pH dependence.

In a previous study of Mn^{2+} uptake, a concentration dependence was demonstrated, with a half-maximal rate at 1 mM (ref. 2). The uptake was measured, however, in the presence of succinate buffer so that a large fraction of the Mn^{2+} was present in the medium in the form of a succinyl complex. In the present study, carried out in the absence of buffer, the concentration dependence for Mn^{2+}, Co^{2+}, and Zn^{2+} was difficult to determine because most of the available cation was taken up in an exceedingly short time. In the case of Co^{2+}, a series of short-term experiments indicated that the midpoint of the saturation curve may be less than 0.01 mM. With Ni^{2+}, on the other hand, the exact concentration dependence was readily demonstrable in both starved and pretreated cells (Fig. 4). The K_m is about 0.5 mM in each case, but the v_{max} is about 5 times higher in cells pretreated with phosphate and glucose.

In pretreated cells, pairs of divalent cations showed mutual inhibition of uptake

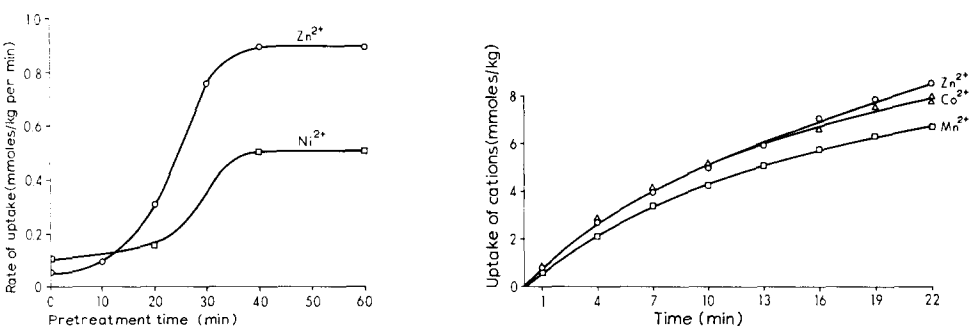

Fig. 1. The effect of pretreatment with phosphate and glucose on the subsequent rate of uptake of Ni^{2+} and Zn^{2+}. The cells were pretreated with potassium phosphate (20 mM (pH 5) in the case of Ni^{2+} and 33 mM Sörensen phosphate buffer (pH 6.0) in the case of Zn^{2+}) *plus* glucose (0.25 M) at 37° for the stated times, then washed, followed by measurements of the rates of cation uptake at 22° and pH 5.5. The Zn^{2+} and Ni^{2+} concentrations were 0.5 mM. Glucose was 0.1 M in the experiment with Ni^{2+} and absent in the experiment with Zn^{2+}. The yeast concentrations were 100 mg/ml for Ni^{2+} uptake and 25 mg/ml for Zn^{2+}.

Fig. 2. Time course of uptake of Mn^{2+}, Co^{2+} and Zn^{2+} by cells pretreated with phosphate and glucose. The cells were pretreated with 33 mM Sörensen phosphate buffer (pH 6) and 0.5 M glucose for 1 h, then washed. During the measurement of uptake, the cation concentrations were 0.25 mM, the pH was 5.5 and the temperature 22°. No substrate was added. The yeast concentration was 25 mg/ml.

in every case, but a precise quantitative estimation of the degree of competitive inhibition was difficult to ascertain because of the afore-mentioned technical difficulty of determining rates of uptake at cation concentrations of the order of 0.01 mM or less. In starved cells in which the rates of uptake were very low, anomalous behavior of certain pairs was observed. Thus the uptake of Zn^{2+} was stimulated by Co^{2+} and that of Co^{2+} was stimulated by Zn^{2+}, but both Co^{2+} and Zn^{2+} uptakes were inhibited by Ca^{2+} (Fig. 5). A more precise kinetic analysis of the inhibitory action, in this case of 1 mM Ni^{2+} on Co^{2+} uptake, is demonstrated in Fig. 6. The data are plotted according to the reciprocal form of the Michaelis–Menten equation:

$$1/v = \frac{1}{v_{max}} + \frac{K_m}{v_{max}[S]}$$

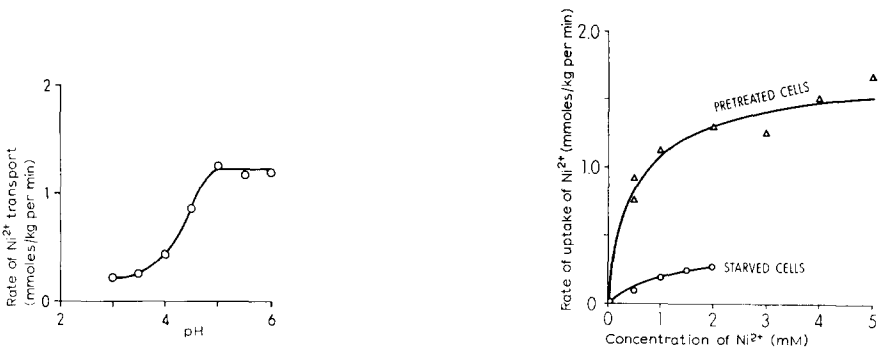

Fig. 3. The pH dependence of Ni^{2+} uptake in pretreated cells. The cells were pretreated with phosphate and glucose as in Fig. 1. Ni^{2+} concentration, 0.5 mM; glucose concentration, 0.2 M; yeast concentration, 25 mg/ml; temperature, 22°.

Fig. 4. The concentration dependence of the uptake of Ni^{2+} in starved and pretreated cells. Pretreatment with phosphate and glucose as in Fig. 1. Glucose concentration, 0.1 M; pH 5; temperature, 22°; yeast concentration, 25 mg/ml.

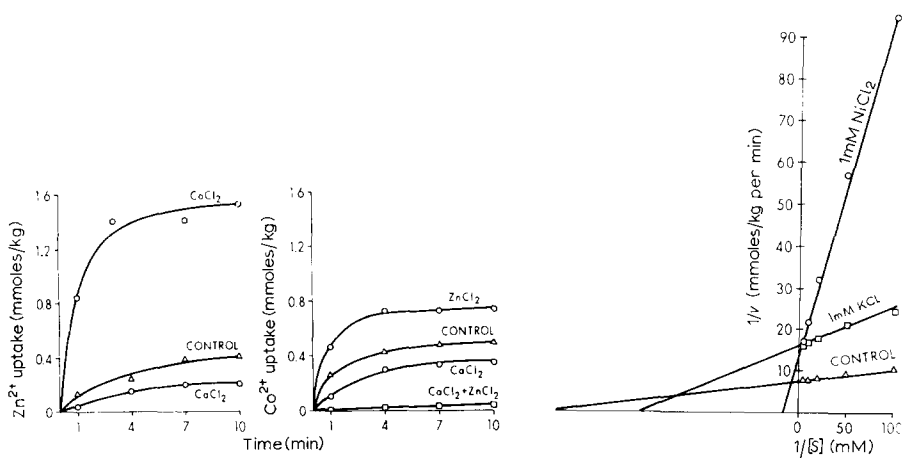

Fig. 5. The effect of Zn^{2+} and Ca^{2+} on Co^{2+} uptake, and of Co^{2+} and Ca^{2+} on Zn^{2+} uptake in starved cells. Cation concentrations, 0.5 mM; pH 5.5, temperature, 22°; yeast concentration, 25 mg/ml.

Fig. 6. The kinetics of inhibition of Co^{2+} uptake by Ni^{2+} and by K^+. Ni^{2+} and K^+ concentrations, 1 mM; glucose concentration, 0.1 M; pH 5.5; temperature, 22°; yeast concentration, 25 mg/ml.

where v is the initial rate of uptake, $[S]$ is the Co^{2+} concentration, v_{max} is the maximal rate and K_m is the Michaelis constant. The K_m for the control data cannot be accurately determined because of the low slope and the long extrapolation. The v_{max} is, however, reasonably precise, 0.08 mmole/kg per min. The inhibitory effect in this case is approximately that of a competitive inhibitor (the intercept would be identical for a strictly competitive effect). In contrast, the presence of 1 mM K^+ results in a definite but smaller inhibition that is predominantly non-competitive.

The uptake of cations must be compensated by an equivalent uptake of anions or by an outflux of cations in order that electroneutrality be preserved. In the case of K^+ transport, for example, uptake is compensated by an efflux of H^+ (ref. 10) or, in Na^+-loaded cells, by an efflux of Na^+ (ref. 11). During uptake of divalent cations, into starved yeast without glucose, no constant increase in excretion of H^+ was detectable, but K^+ efflux, which is normally very small, was increased considerably. In quantitative terms the ratio of extra K^+ excretion to Co^{2+} uptake was 2.2/1.0 (average of four experiments), giving approximate equivalence. In Na^+-loaded cells, approx. 2 Na^+ were excreted for each Co^{2+} taken up.

DISCUSSION

The divalent cations, Ni^{2+}, Co^{2+} and Zn^{2+}, like all other cations tested, bind reversibly to surface anionic sites. But in addition they can be transported into a non-exchangeable pool of the yeast cell. In this respect they resemble Mg^{2+} and Mn^{2+}. Indeed, the specific transport system previously described for Mg^{2+} and Mn^{2+} (ref. 2) seems to be the system responsible for the transport of Co^{2+}, Zn^{2+} and Ni^{2+}, a conclusion based on direct comparisons of Mn^{2+} and Co^{2+}, Ni^{2+} and Zn^{2+} transport; on the apparent competitions between pairs of cations; on the dependence on a common energy source, fermentation; and on the unique dependence on a pretreatment period during which phosphate uptake occurs. The exact affinities of Mn^{2+}, Zn^{2+} and Co^{2+} for the system are difficult to determine but the K_m's are probably less than 0.01 mM. That of Ni^{2+} is higher, 0.5 mM. The affinity series, based on the present and previous studies[2] is probably Mg^{2+}, Co^{2+}, $Zn^{2+} > Mn^{2+} > Ni^{2+} > Ca^{2+} > Sr^{2+}$.

The nature of the transport system is not clearly understood, but it has many of the qualities of an active transport system: saturability, specificity, dependence on metabolism, asymmetry (high influx and very low efflux), and competition of pairs of cations. It differs from other transport systems inasmuch as its level is decreased during starvation, but can be restored by allowing cells to absorb inorganic phosphate. The inward transport of phosphate through the membrane apparently results in the synthesis of a component essential for divalent cation transport[3].

A relatively non-specific form of divalent cation transport by means of the "K-carrier" has also been reported[12,13]. Such transport is quite distinct from that reported here. The affinity for divalent cations is very low with K_m's of the order of 100 mM, with the uptake almost completely and competitively blocked by low concentrations of K^+, with the uptake balanced by H^+ secretion, and with distinct aerobic–anaerobic differences. In contrast, the transport system reported here has a very high affinity for certain divalent cations; with a small, non-competitive inhibition by K^+; with uptake balanced by K^+ efflux; and with no aerobic–anaerobic

differences. Furthermore, the level of divalent-carrier is dependent on pretreatment with phosphate, whereas the level of the K^+-carrier is not.

Large quantities of divalent cations can be transported into the cell in short periods of time, especially into cells pretreated with phosphate and glucose (over 7 mmoles/kg in 20 min). Even in well-starved cells, a significant uptake occurs, especially in the presence of glucose (except for zinc). The uptake in the case of Ni^{2+} can be large enough to induce a specific block in the alcohol dehydrogenase reaction, leading to a partial inhibition of fermentation[8].

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